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Sex and fat—Can one factor handle both?

It has become increasingly evident that, in addition to determining female sex development, estradiol plays additional important roles in both males and females, not the least of which is regulation of energy homeostasis. A new study by D'Eon et al. (2005) now further highlights this role.

The concept of estradiol as the female sex hormone and testosterone as the male sex hormone has undergone a considerable makeover in recent years. This is based on the realization that both hormones coexist in males and females and can have numerous roles, many of which have nothing to do with sex or reproduction. Thus, it has recently been shown, in studies with genetically modified mice and in patients with natural mutations in the human *aromatase* and *ERα* genes (Oz et al., 2000; Carani et al., 1997; Smith et al., 1994), that estrogen plays just as important a role in the maintenance of healthy bones in males as it does in females. Sex hormones are also involved in the cardiovascular system and in brain function. Additionally, it has become increasingly apparent that sex hormones perform key roles in energy homeostasis. It is now quite well documented that systemic loss of estrogen at menopause is associated with increased adiposity, which can be prevented by estrogen replacement. Studies in mice bearing inactivating mutations in either aromatase, the enzyme responsible for estrogen biosynthesis (ArKO), or in the ERα estrogen receptor (ERKO) have shown that these mice develop a full-blown metabolic syndrome characterized by increased visceral and gonadal fat, hyperleptinemia, hyperinsulinemia, and decreased physical activity (Heine et al., 2000; Jones et al., 2000; Misso et al., 2003). Moreover both of these models exhibit a sexually dimorphic hepatic steatosis that, interestingly, is male specific (Hewitt et al., 2004). In the case of the ArKO mice, it was shown that the increase in visceral adiposity was primarily due to an increase in the expression of lipoprotein lipase (LPL) rather than increased de novo synthesis of fatty acids. Estradiol replacement produced a dramatic decrease in adiposity, a decrease in the expression of LPL and leptin, and an increase in adipose tissue fatty-acid β-oxidation. The male-specific hepatic steatosis was shown to be accompanied by an increase in the expression of fatty-acid synthase (FAS) and acetylCoA carboxylase-1 (ACC-1), a phenotype that could be reversed by estradiol replacement. A muscle phenotype of the ArKO mice was also suggested by decreased physical activity, a decrease in lean body mass and rate of glucose oxidation, and an increase in muscle triglycerides. A complicating factor in these studies is the fact that both the ArKO and the ERKO models show elevated circulating androgens. This issue was obviated in a recent publication by D'Eon et al. in the *Journal of Biological Chemistry* (D'Eon et al., 2005). The authors employed ovariectomized mice as their study model. Since it is a well-established dogma that the adrenals of rodents do not secrete androgenic steroids, it is reasonable to conclude, although this was not in fact determined, that the circulating testosterone level in these animals is extremely low. The authors also employed a pair-feeding paradigm in order to avoid the issue that ovariectomy induces hyperphagia in rodents, which is itself ameliorated by estradiol replacement. Thus they were able to define how estrogen protects against increased adiposity in this model independently of differences in energy intake.

Following 60 days of replacement with estradiol in the form of implants, it was found that estrogen treatment decreased total adiposity as well as adipocyte size in the visceral fat depots but not in subcutaneous fat. Circulating leptin and resistin were both decreased, as was circulating adiponectin. In the adipose tissue, estrogen replacement resulted in a decrease in the expression of factors and enzymes, such as LPL, LXRα, SREBP-1c, FAS and ACC-1, involved in triglyceride synthesis. Employing isolated adipocytes, it was shown that the estradiol-treated mice had higher isoproterenol-stimulated lipolysis as determined by glycerol release. Western analysis of blots for perilipin and HSL expression revealed that this was due to higher perilipin expression in cells from the estradiol-treated mice, as there was no significant difference in HSL protein expression.

The muscle tissue of these animals was also investigated in this study. In muscle from the ovariectomized mice, estrogen treatment resulted in decreased expression of SREBP-1c and its downstream targets, FAS and ACC-1. Estrogen treatment also resulted in an increased expression of PPARγ and its downstream targets such as UCP2 and 3, ACOX, and PDK4. Thus, as in the fat, estrogen administration resulted in decreased fat synthesis and increased energy utilization in muscle. Since AMP kinase is implicated in lipid homeostasis in muscle, the effect of estrogen treatment on AMP kinase activation was determined employing Western blots of phosphorylated AMP kinase (T172). AMP kinase, by regulating intracellular signals, acts as a fuel gauge regulating fat oxidation, fatty-acid synthesis, and glucose uptake. Activation of AMP kinase results in inactivation of ACC, thus preventing the synthesis of malonyl CoA, a metabolite necessary for triglyceride synthesis. This in turn increases carnitine palmitoyl transferase-1 activity and allows for long-chain fatty-acid transport into the mitochondria for oxidation (Figure 1; reviewed in Kahn et al., 2005). It was found that muscle from the estrogen-treated mice had higher levels of phosphorylated AMP kinase when normalized for total kinase protein. In a further attempt to determine if there was a direct action of estradiol on the muscle cells, C2C12 myocytes in culture were employed and estrogen at different concentrations was added to the culture medium. Estradiol caused increased phosphorylation of AMP kinase within 5 min in a dose-dependent fashion, and this phosphorylation was fully inhibited by ICI 182780, a pure estrogen receptor antagonist. However, a feature of this experiment that is difficult to reconcile is that there appeared to be little change in the phosphorylation of AMP kinase at estrogen levels of 10 and 100 nmol. This effect was only seen at higher levels,

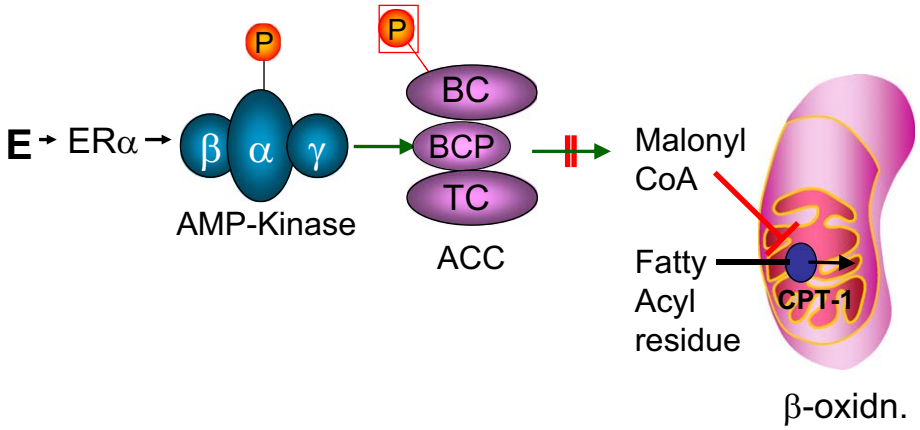

Figure 1. Proposed role of estrogen in the activation of the AMP kinase pathway regulating fatty-acid β-oxidation

namely 1 μmol and 10 μmol estradiol. Since the estrogen receptor is fully saturated at concentrations of 1 nmol, it is hard to imagine that this was an ERα- or ERβ-mediated event, although this was suggested by the blockade by the estrogen receptor inhibitor. One possible speculation is that there could have been an extremely rapid metabolism of the estradiol by these cultured CTC12 myocytes, although this had not been tested.

This study is a useful addition to the previously published work employing genetically modified mouse models of estrogen insufficiency. Based on their observations, the authors suggest that estradiol reduces adiposity by the enhancement of pathways that promote fatty-acid oxidation in muscle, inhibition of fat storage in adipose tissue, liver, and muscle, and enhanced rates of adipocyte lipolysis. In addition, they suggest that estradiol, through upregulation of PPARγ expression, acts in muscle to alter fuel partitioning and oxidative capacity and thus enhance muscle oxidation. Whereas these results can be explained by classical genomic actions of estradiol, the in vitro studies suggest that estradiol could also act in a rapid nongenomic manner to phosphorylate, and thus activate, AMP kinase in muscle. Although this reported action of estradiol is intriguing, the fact that high concentrations of estradiol were required in order to achieve this effect leaves the door open to other interpretations.

This is an important issue that should be clarified since it is now becoming apparent from these, and other studies, that not only estradiol but also testosterone play important roles in the regulation of energy homeostasis. Humans with natural mutations in aromatase develop a metabolic syndrome (Maffei et al., 2004), but hypogonadal men also have a strong tendency toward obesity. Moreover, polycystic ovarian syndrome in women, which is characterized by hyperandrogenicity, is frequently also marked by insulin resistance and type II diabetes. These results are confusing and seemingly conflicting, and the issue will only be resolved by careful analysis of animal models such as the one described in this report as well as the use of transgenic mouse models employing tissue-specific knockouts and knockins.

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